Phytochemical Profile and Antioxidant Activity of *Acalypha Wilkesiana* Leaves

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Abstract

Oxidative stress is one of the problems facing in healthcare system and is a vital cause of most deadly diseases. Modern medicine has done much to combat these diseases but most of the drugs have side effects that are also detrimental to the patient and also very expensive. This study aimed to determine the antioxidant potential and also qualitatively and quantitatively evaluate the phytochemicals of Acalypha wilkesiana leaf. The leaves were collected, air-dried and pulverized. The leaves were extracted and screened to determine phytochemicals that are present using a standardized protocol. Flavonoids, saponins and phenolics contents were quantified using spectrophotometric assay. The phytochemicals that are present in the extract were identified using GC-MS while the antioxidant activity of the methanolic extract was determined using DPPH and FRAP assay.From this study, it was observed that alkaloids, phenolics, flavonoids, saponins, tannins, glycosides, steroids, and coumarin were present in methanolic extract while only alkaloids, steroids and coumarin are present in n-hexane extract of the plant. Spectrophotometric assay revealed a total flavonoid, saponin and phenolic content to be 29.988 ± 0.089(QE/mg/g), 2.3 4± 0.042 (mg/g), 81.627 ± 0.321(GAE/mg/g) respectively. The GC-MS analysis revealed 16 compounds. The first compound identified is 2-Propanone with retention time of 5.98 minutes while the last is 9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, trans- with retention time of 40.00 minutes. Among the compounds that were identified, Di-n-octyl phthalate (33.64 minutes) and 7-Tetradecene (11.75 minute) showed the highest percentage composition of 12.97% and 12.52% respectively. The results of the DPPH and FRAP assays of Methanolic extract shows the DPPH radical scavenging percentage of 47.21% and the FRAP value of 113.61 µmol Fe^{2+}/g with IC_{50} value of 24.21 µg/mL and IC_{50} of 120.2 respectively. This shows that Acalypha wilkesiana leaves have potential as natural antioxidant source.

Key words*: Oxidative-stress; Phytochemicals; GC-MS; Antioxidant; Acalypha wilkesiana, leaves*

Introduction

Human exposure to exogenous substances such as radiation, toxins, chemicals, environmental pollutants, as well as physical stress and endogenous metabolic processes, leads to the production of reactive oxygen species (ROS), including superoxide, hydroxyl radicals, peroxide radicals, and hydrogen peroxide [1]. These ROS are responsible for cellular damage through oxidative stress. Research has shown that these reactive species contribute to several life-threatening diseases, including cancer, inflammation, cardiovascular diseases, and neurodegenerative disorders [2]. Although modern medicine has made significant strides in combating these diseases, many treatments come with side effects that are harmful to patients and often carry high costs. As a result, there is a growing need for alternative treatments that are more affordable, effective, and exhibit minimal or no side effects [3].

Acalypha wilkesiana, a member of the Euphorbiaceae family that is commonly known as copper leaf, Joseph's coat, or fire dragon [4]. In Yoruba, it is referred to as "Aworoso." This popular ornamental plant is native to Fiji and the surrounding islands in the South Pacific but has since spread to tropical regions of Africa, America, and Asia. *Acalypha wilkesiana* is renowned for its wide range of medicinal properties [5]. Studies have identified its biological activities, including antimicrobial, antioxidant, antidiabetic, anti-inflammatory, pupicidal, larvicidal, hepatoprotective, anticancer, leishmanicidal, antihypertensive, anti-hyperglycemic, anti-venom, analgesic, antiemetic, anthelmintic, laxative, expectorant, diuretic, post-coital, anti-fertility effects, and wound healing [6,]. In Nigeria, the therapeutic value of *Acalypha wilkesiana* is widely recognized. In Southern Nigeria, its leaf extract or boiled decoction is used in traditional healthcare to treat fungal skin infections, hypertension, gastrointestinal disorders, and diabetes mellitus [7]. The leaf poultice is also employed for treating headaches, swellings, colds, and malaria [8]. Additionally, *Acalypha wilkesiana* ointment has been reported to treat fungal skin diseases [9, 10]. This indicates that the plant has significant antifungal potential, making it a promising medicinal resource. Furthermore, *Acalypha wilkesiana* has been shown to be beneficial for cardiovascular and diabetes-related diseases. In a study by Ikewuchi and Ikewuchi, the aqueous extract of the plant demonstrated a reduction in both blood cholesterol and blood sugar levels in a rat model, supporting its traditional use in treating cardiovascular issues [11]. Moreover, Gotep et al., conducted an *in vitro* antimicrobial screening of the ethanol extract of *Acalyphawilkesiana*, revealing varying degrees of antimicrobial activity against pathogens such as *Staphylococcus aureus, Yersinia enterocolitica, Escherichia coli, Salmonella typhii, Pseudomonas aeruginosa, and Klebsiella aerogenes*[12]. This research aims to evaluate the antioxidant potential of *Acalypha wilkesiana* leaf extract and quantify its flavonoid, saponin, and phenolic content.

2.0 Materials and Methods

Materials

All reagents used are of analytical grade and were utilized without further purification. n-hexane, Methanol, Nitric acid, Sulphuric acid, Ammonia, Distilled water, Sodium hydroxide, Chloroform, Ferric Chloride, Hydrochloric acid were from Merck.

2.1 Sample Collection

The plant leaves of *Acalypha wilkesiana* was collected from Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria. The plant was identified in the Department of Pure and Applied Biology by Prof. A. T. J. Ogunkunle. A voucher specimen was deposited in their

herbarium and specimen number of LHO 760 was attached to it. The plant sample was washed and air dried at room temperature for a month. The dried plant sample was then pulverized into a fine powder using an electric blender, weighed and stored for further use.

2.2 Extraction process

Cold extraction was carried out on the air-dried and pulverized leaves of *Acalypha wilkesiana* using a soxhlet extractor.

500g of pulverized plant sample was soaked with 2.5mL n-hexane for seventy two hours interval and then filtered. The residue was soaked with 2.5mL methanol for seventy two hours interval as well and then filtered. The two separate solvent extracts were concentrated by evaporation using rotary evaporator.

2.3 Phytochemical Screening

The phytochemical components of leaves extracts were evaluated using a standardized protocol described by Shaikh and Patil., (2020) and Adepoju*et al* (2024) [13, 14]. Saponins, alkaloids, flavonoids, tannins, coumarin, steroids, terpenoids, glycosides and phenol were among the phytochemicals investigated.

2.4 Gas Chromatography-Mass Spectrometric Analysis

GC-MS analysis was carried out at the Analysis Research Laboratory, Afe Babalola University Ado-Ekiti, Nigeria. GC-MS (Varian 3800/4000 gas chromatograph mass spectrometer) analysis was carried out under the following conditions: carrier gas: nitrogen; column: HP-5MS capillary column (30 m length, 0.25 mm ID, 0.25 μm thickness); Oven temperature: 50°C (4 min hold) increased to 280°C at a rate of 10°C/min (10 min hold), MS source temperature: 260°C, Injection Temperature: 220°C, Carrier Gas Flow Rate: 1.0 mL/min, Split ratio: 10:1, Injection mode: Manual, Injection volume: 1.0 μL. For the ionization of electrons, 70 eV was used. The scan range was 50-500 m/z. The mass of the compounds and the fragments recorded were matched with NIST 08 and Wiley 8 libraries to identify probable compounds present in the sample. Other research articles were cross-referred to the structure and activities of the detected compound. Using computer searches on a NIST Ver. 08 MS data library and comparing the spectrum obtained through GC-MS, compounds present in the plant samples were identified. Interpretation on mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were confirmed.

2.5 Determination of Total Flavonoid Content

Aluminium colorimetric method was used to determine the total flavonoid content of the methanolic extract of *Acalypha wilkesiana* leaves. 1 ml of the crude methanolic leaf extract was dissolved in 5 ml of 50% methanol and 1 ml of this solution was added to 0.7 ml of 5% (w/w) $NaNO_2$, and 10 ml of 30% (v/v) ethanol. This mixture was stirred for 5 mins and 0.7 ml of 10% $AlCl_3$ (w/w) was then added to it and stirred for 6mins. 5 ml of 1mol/l NaOH was added to the mixture and diluted to 25 ml with 30% (v/v) ethanol. The mixture was allowed to stand for 10 mins and the absorbance measured at 500 nm using a UV spectrophotometer. Quercetin was used

as the standard and the total flavonoid content was expressed as quercetin equivalent in mg/g extract [15].

2.6 Determination of Total Phenolic Content

The total phenolic content of the crude methanolic extract of *Acalypha wilkesiana* was determined using the Folin-Cincalteau method. 5 mg of themethanolic leaf extract was weighed and dissolved with 5 ml of 50% methanol using a vortex mixer. 0.5 ml of this solution was pipette into a test tube and 3.5 ml of distilled water, 0.25 ml Folin-Ciocalteau reagent was added to it. It was left to incubate for 8 minutes at room temperature. Then 1 ml of 20% Na_2CO_3 was then added and left to incubate for 2 hrs. The absorbance was measured at a wavelength of 765 nm against a blank using a UV spectrophotometer. Gallic acid was used as the standard and the total phenolic content of the extract expressed in mg Gallic acid equivalents/mg extract [16].

2.7 Determination of Total Saponin Content

Crude methanolic extract of 1ml was placed in a conical flask and 10 cm^3 of 20% aqueous ethanol was added to it and heated over a hot water bath for 4hrs with continuous stirring at about 55^0C. The mixture was filtered and extracted three times with 10 ml of 20% aqueous ethanol. The extracts was then combined and the volume reduced to 4 ml using a water bath at about 90°C. The concentrated extract was transferred into a 250 ml separating funnel, and then 20 ml diethyl ether was added and shaken vigorously. The aqueous layer was recovered and the process was repeated. 10 ml of n-butanol was then added to the aqueous layer. The butanol fraction was recovered and washed twice with 10 ml of 55% NaCl. The solution was heated in a water bath to evaporate off the butanol. After evaporation, the sample was dried in an oven to constant weight to recover saponin.

To prepare the recovered saponin for UV measurement, a fresh solution of vanillin-acetic acid (5% w/v, 0.2 ml) solution was prepared and 0.8 ml perchloric acid was then added and kept at 70°C for 15 minutes. The solution was cooled on ice for 20 seconds before adding 5 mlglacial acetic acid. The solution was scanned at 550 nm using a Cecil CE7200 UV spectrophotometer. The standard used was GinsenosideRbl as predetermined graphically by [17].

2.8 Determination of 1,1, dipheny-2-picrylhydrazyl (DPPH) Radical Scavenging Activities

The DPPH radical scavenging assay was performed using 1,1 diphenyl-2-picrylhydrazyl (DPPH) according to the method described by Brand-Williams *et al*.with some modifications. Briefly, five different concentrations of the studied plant extracts (20, 40, 60, 80, and 100 µg/ml) were prepared in methanol (analytical grade). The same concentrations were also prepared for L-ascorbic acid, which was used as a standard antioxidant. 1 ml of each studied extract was transferred into a clean test tube into which 0.5 ml of 0.3 mM DPPH in methanol was added. The mixture was shaken and left to stand in the dark at room temperature for 15 minutes. Blank solutions comprising of the studied extract solutions (2.5 ml) and 1 ml of methanol were used as baseline.

The negative control comprised 2.5 ml of DPPH solution and 1 ml of methanol, while L-ascorbic acid at the same concentrations as the studied extracts was used as the positive control. After incubation in the dark, the absorbance values were measured at 517 nm using a spectrophotometer. The experiments were performed in triplicate. The DPPH radical scavenging activity was estimated using the equation described by Brand-Williams *et al*. [18].

$$\% \text{ Radical scavenging activity} = \frac{A_c - A_s}{A_c} \times 100,$$

where A_s is the absorbance of the sample, and A_c is the absorbance of the control.
The half maximal inhibitory concentration (IC_{50}) of the extracts was computed from a plot of percentage DPPH free radical inhibition versus the extract concentration.

2.9 Determination of Ferric Reducing Antioxidant Power Assay

The reducing power of the extracts was determined according to the method described by Oyaizu with some modifications. Briefly, five different concentrations of methanolic extracts (20, 40, 60, 80, and 100 µg/ml) and L-ascorbic acid at same concentrations were mixed with 2 ml phosphate buffer (0.2 M, pH 6.6) and 2 ml of 1% potassium ferricyanide ($K_3Fe(CN)_6$). The mixture was incubated at 50°C for 20 minutes. Then, 2 ml of 10% trichloroacetic acid (TCA) was added, and the mixture was centrifuged at 1000 revolutions per minute (rpm) for 10 min. The supernatant (2 ml) was aspirated and mixed with 2 ml of distilled water and 1 ml of 0.1% ferric chloride ($FeCl_3$). In each case, the experiment was performed in triplicate. Afterward, the absorbances were measured spectrophotometrically at 700 nm using a UV-vis spectrophotometer and recorded.

3.0 Results and Discussion

In most phytochemical studies, determining the weight of the extract is essential for calculating the percentage yield of the phytochemicals present in the plant. In this study, the percentage yield of the crude extracts from the plant *Alkalypha wilkesiana* leaves was determined and is provided in Table 1. The yield of the methanol extract suggests a significant presence of large quantity of polar compounds.

Table 1 Percentage yield of n-hexane and methanolic extracts of *Acalypha wilkesiana* leaves

Extracts	Yield (%)
n- hexane	0.61
Methanol	3.63

3.1 Phytochemicals screening

Identifying the type or class of phytochemicals is crucial, as it serves as an initial step in identifying specific plant chemicals. Phytochemical screening of the crude extracts from *Acalypha wilkesiana* leaves indicated the presence of alkaloids, phenol, flavonoids, saponins, tannins, glycosides, steroids,coumarins while terpenoids was absent in the methanol extract. In contrast, the n-hexane extract contained only alkaloids, steroids and coumarins while phenol, flavonoids, saponins, tannins, glycosides and terpenoids were absent (Table 2).The presence of various phytochemicals in methanolic and n-hexane extracts can be attributed to the solvent's polarity and its ability to dissolve specific classes of compounds [19]. Phytochemicals found in plants have demonstrated various biological and pharmacological activities, including antimicrobial, anti-inflammatory, anticancer, and antioxidant properties [20].

Table 2 Phytochemicals Screening of n-Hexane and Methanolic extracts of *Acalypha wilkesiana* leaves

Extracts	Alk	Tan	Sap	Flav	Gly	Terp	Phen	Coum	Ste
n-hexane	++	-	-	-	-	-	-	+	++
Methanol	+++	++	++	++	++	-	+++	+++	+

KEY: Alk – alkaloids, Tan – tannins, Sap – saponins, Flav – flavonoids, Gly – glycosides, Terp – terpenoids,
Phen – phenol, Coum – coumarins, Ste – steroids, +++ = highly present, ++ = moderately present, + = slightly
present, **-** = absent

In this study, alkaloids, phenol, flavonoids, saponins, tannins, glycosides, steroids and coumarins were detected in *Acalypha wilkesiana* leaves extracts, which is consistent with the findings of (Michea*et al*., 2023). However, Daniel and Effiong (2019) reported the absence of coumarins, while Mendame *et al*. (2022) noted the absence of saponins [21-23]. The observed discrepancies in the phytochemical screening of the leaf extracts may be attributed to variations in soil, climate, and environmental conditions. Despite these differences, the findings of these researchers align with the results of this study regarding the presence of alkaloids, phenols, flavonoids, tannins, glycosides, and steroids, which may contribute to the plant's biological activities. The presence of tannins suggests the plant's potential as an antidiarrheal and antihemorrhagic agent, while alkaloids indicate its use in detoxification and hypertension management [24]. Flavonoids are known to protect cells from oxidative damage, and phenolic compounds serve as important antioxidants that may help prevent carcinogen formation [25].

Saponins are recognized for their antifungal and antiviral properties, as well as their ability to lower cholesterol and enhance mucosal drug absorption [26]. Steroids are characterized by lowering cholesterol levels, and certain hormones like phytoestrogens, which may mimic the effects of estrogen, and coumarins have anti-cancer, anti-inflammatory and antimicrobial properties. Some coumarins are used in medicine to prevent blood clots, while others are used in the fragrance industry [27].

3.2 Quantification analysis

The total phenolic, flavonoid, and saponin contents of the crude methanolic extract from *Alkalypha wilkesiana* leaves were quantified using a colorimetric method. The methanolic extract demonstrated a high phenolic content of 81.627 ± 0.321 mg gallic acid equivalent per gram of extract, a total flavonoid content of 29.988 ± 0.089 mg quercetin equivalent per gram of extract, and a total saponin content of 2.34 ± 0.042% (Table 3). These results indicate that the extract is rich in phenolic compounds and flavonoids, but relatively low in saponins. This variation may be attributed to the specific plant species, the extraction method used, and the distribution of these compounds within the plant material.

Table 3 Quantities of some selected phytochemicals in the methanolic extract

Methalonic extract	Total Phenolic contect (GAE/mg/g)	Total Flavonoid content (QE/mg/g)	Total Saponin content (%)
	81.627±0.32	29.988±0.09	2.34±0.04

GAE = Gallic acid equivalent; QE = Quercetin equivalent

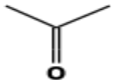
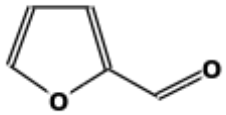
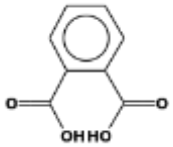
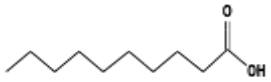
Phenolic compounds are known for their potent antioxidant properties, helping to neutralize free radicals and reduce oxidative stress, which can protect cells and tissues from oxidative damage [28]. This is particularly beneficial in preventing diseases such as cancer, heart disease, and neurodegenerative disorders. Additionally, phenolics exhibit anti-inflammatory effects by inhibiting pro-inflammatory cytokines and enzymes like cyclooxygenase (COX) and lipoxygenase (LOX), making them useful in managing inflammatory conditions such as arthritis and cardiovascular diseases [29]. They also show antimicrobial activity by interacting with microbial cell membranes, inhibiting enzyme activity, and disrupting microbial growth, making them effective against various bacteria, fungi, and viruses [30].

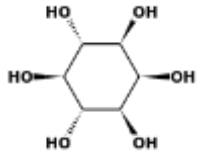
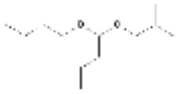
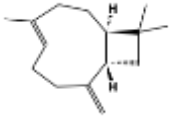
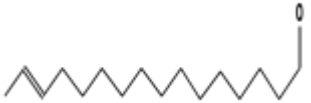
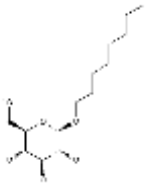
Flavonoids, on the other hand, are reported to possess a wide range of biological and pharmacological activities, including antioxidant [31, 32], anti-inflammatory [33], antidiabetic [34],cancer-preventive [35, and cardiovascular effects [36]. Therefore, the use of Alkalypha wilkesiana leaves in traditional medicine is recommended due to their high phenolic content and moderate flavonoid levels, both of which are associated with various health benefits.

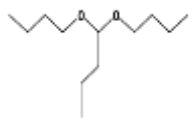
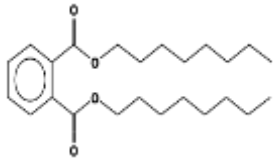
3.3 GC-MS analysis

Gas chromatography – Mass spectrometry (GC-MS) is a unique analysis technique used for identification and quantification which is limited to analytes that are not only volatile and thermally labile but can also withstand the harsh partitioning conditions of the gas chromatograph. The instrument can ascertain the compounds that are present in each extract of the plant. Gas chromatography – Mass spectrometry revealed 16 compounds and identified them from the NIST library coupled with the GC-MS. The first compound identified is 2-Propanone with retention time of 5.98 minutes and the last been identified is 9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, trans- with retention time of 40.00 minutes. Among the compounds that were identified, Di-n-octyl phthalate (33.64 minutes) and 7-Tetradecene (11.75 minute) showed the highest percentage composition of 12.97% and 12.52% respectively. Thereafter 3-Eicosene (E)- (31.58 minutes), Myo-Inositol (12.61 minutes), 1,2-Benzenedicarboxylic acid (8.50 minutes) and 2-Propanone (5.98 minutes)showed the percentage composition of 11.74%, 10.90%, 9.57% and 8.42% as well.Other compounds identified were Furfural (6.75 minutes), n-Decanoic acid (11.42 minutes),3-Ethoxycarbonyl-5-hydroxytetrahydropyran-2-one (15.50 minutes), 1-Butoxy-1-isobutoxy-butane (19.50 minutes),Caryophyllene (21.20 minutes), E-14-Hexadecenal (22.50 minutes),Octyl beta-D-glucopyranoside (24.68 minutes), Butane, 1,1-dibutoxy- (27.41 minutes) and Di-tert-butyl decarbonate (28.00 minutes) as shown in Figure 1 and Table 4

Table 4. GC-MS analysis of methanolic leaf extract

Peak #	RT	Compound Detected	Mol. Formula	MW	Peak Area %	weight Composition %	m/z	Structures
1	5.98	2-Propanone	C_3H_6O	58	25.76	8.42	41, 43, 58	
2	6.75	Furfural	$C_5H_4O_2$	96	8.33	5.51	67, 95, 96	
3	8.50	1,2-Benzene dicarboxylic acid	$C_8H_6O_4$	166	9.85	9.57	43,78, 166	
4	11.42	n-Decanoic acid	$C_{10}H_{20}O_2$	172	1.51	2.11	41,60, 172	
5	11.75	7-Tetradecene	$C_{14}H_{28}$	196	11.36	12.52	43,69, 196	

6	12.61	Myo-Inositol	$C_6H_{12}O_6$	180	9.46	10.90	43,73, 180	
7	15.50	3-Ethoxy carbonyl-5-hydroxy tetrahydro pyran-2-one	$C_8H_{12}O_5$	188	1.14	2.03	43,87, 188	
8	19.50	1-Butoxy-1-Isobutoxy -butane	$C_{12}H_{26}O_2$	202	1.29	3.59	57,73, 202	
9	21.20	Caryophy llene	$C_{15}H_{24}$	204	2.12	3.21	41,93, 204	
10	22.50	E-14-Hexa Decenal	$C_{16}H_{30}O$	238	1.21	2.07	41,55, 238	
11	24.68	Octyl-β-D-glucopyranoside	$C_{14}H_{28}O_6$	292	1.67	1.83	57,60, 292	

12	27.41	1,1-dibutoxy Butane	$C_{12}H_{26}O_2$	202	3.18	4.15	41,57, 202	
13	28.00	Di-tert-butyl decarbonate	$C_{10}H_{18}O_5$	218	2.88	3.98	41,57, 218	
14	31.58	3-Eicosene, (E)-	$C_{20}H_{40}$	280	6.06	11.74	43,57, 280	
15	33.64	Di-n-octyl Phthalate	$C_{24}H_{38}O_4$	390	7.95	12.97	43,140, 390	
16	40.00	acid,(2-phenyl-1,3-dioxolan-4-yl)methyl ester trans-9-Octa decenoic	$C_{28}H_{44}O_4$	444	6.21	5.56	43,91, 444	

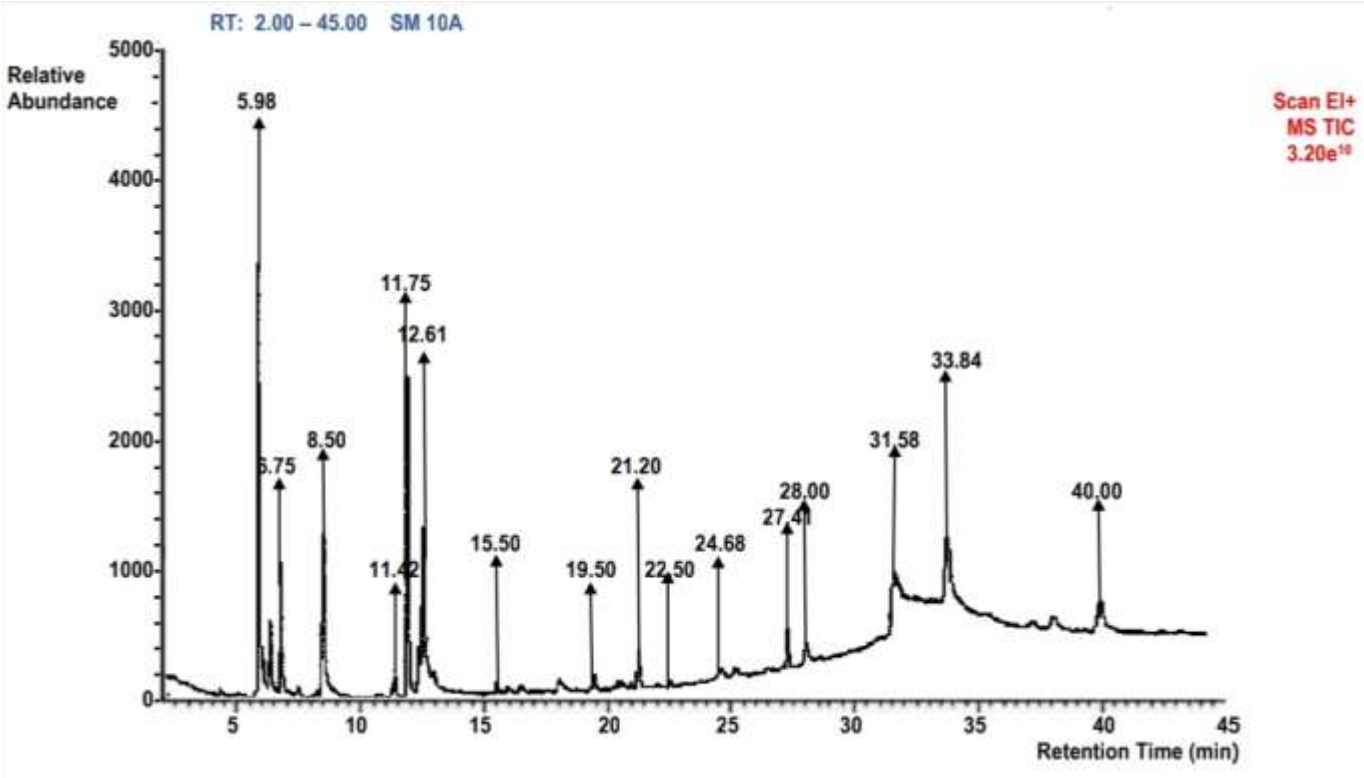


Figure 1: Total ion chromatogram of the methanol leaf extract

The identified classes of compounds include alkenes, alcohols, carbonyls, carboxylic acids, terpenes, ethers, phthalates, and fatty esters. These compounds have been reported to exhibit various biological and pharmacological activities, such as antimicrobial, anti-inflammatory, neuroprotective, antifungal, and antioxidant effects. Di-n-octyl phthalate is commonly used as a plasticizer to increase the flexibility of plastics, but it has been identified as an endocrine disruptor that can interfere with hormonal systems. It may also exhibit cytotoxicity, potentially affecting cellular development and reproduction [37]. 7-Tetradecene is known for its defensive and signaling roles and can act as a natural insect pheromone [38]. 3-Eicosene, a derivative of unsaturated fatty acids found in plant and animal oils, has been reported to help prevent oxidative stress-related diseases [39, 40]. Myo-inositol has been studied for its potential to manage polycystic ovary syndrome (PCOS) and improve insulin sensitivity, in addition to demonstrating neuroprotective and antidepressant-like effects [41, 42]. Furfural shows antioxidant and anti-inflammatory properties, with its derivatives being explored for use as natural preservatives or treatments for oxidative stress-related diseases [43]. n-Decanoic acid plays a role in energy production and membrane structure, exhibiting antimicrobial, anti-inflammatory, and antidiabetic effects, and shows promise in managing metabolic disorders [44, 45]. Octyl beta-D-glucopyranoside has mild antimicrobial and antifungal properties, attributed to its ability to disrupt cell membranes [46].

3.4 Antioxidant activity

The antioxidant activity of plant extracts is a crucial area of research, particularly in relation to their potential therapeutic and preventive applications in diseases associated with oxidative stress. In the present study, the antioxidant properties of a methanolic extract were evaluated using two commonly used assays: the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity and the FRAP (Ferric Reducing Antioxidant Power) assay. These assays provide valuable insight into the extract's ability to neutralize free radicals and donate electrons to reduce oxidized species, respectively(Fernandez-Arroyo et al., 2011; Krishnaiah et al., 2011). In this analysis, the results of these assays demonstrate a moderate to good antioxidant activity of the methanolic extract, providing a deeper understanding of its potential as a natural antioxidant source (Table 5).

Table 5 Antioxidant capacity of methanolic extract

Antioxidant	Composition	IC_{50}
DPPH (µg/mL)	47.22	24.21
FRAP (µg/mL)	113.61	120.2

DPPH = 1,1, dipheny-2-picrylhydrazyl;FRAP = Ferric Reducing Antioxidant Power Assay

The DPPH assay is one of the most widely used methods to evaluate the radical scavenging capacity of antioxidants. DPPH is a stable free radical that, when exposed to an antioxidant, undergoes a reduction, resulting in a color change from purple to yellow[47]. The extent of this color change is directly proportional to the antioxidant's ability to scavenge free radicals.In the present study, the methanolic extract demonstrated a DPPH radical scavenging percentage of 47.21%, indicating that the extract is capable of neutralizing almost half of the DPPH radicals in the solution. This is a promising result, as it suggests that the methanolic extract contains bioactive compounds with the potential to neutralize harmful free radicals and reduce oxidative stress. The IC_{50} value, which represents the concentration of the extract required to scavenge 50% of the DPPH radicals, was recorded at 24.21 µg/mL. IC_{50} values are commonly used to evaluate the potency of antioxidants: lower IC_{50} values indicate stronger antioxidant activity, as a smaller amount of the substance is required to achieve a 50% inhibition of the free radicals [48].

To provide context, the slightly higher IC_{50} value of the plant extract (24.21 µg/mL) compared to ascorbic acid (20 µg/mL) indicates that ascorbic acid is marginally more effective in inhibiting the target biological activity. However, the difference is minimal and may not be biologically or clinically significant. Despite its slightly higher IC_{50}, the plant extract offers potential advantages, such as synergistic effects and a broader therapeutic profile, which make it an appealing natural alternative. Its complex composition may provide additional health benefits beyond those offered by ascorbic acid alone. Therefore, while the extract might require slightly higher concentrations to achieve similar effects, its multi-target mechanisms and natural origin should not be dismissed in favor of isolated compounds like ascorbic acid.

The free radical scavenging potential of an extract is closely linked to its chemical makeup. Compounds like polyphenols, tannins, alkaloids, and flavonoids are well-known for their potent antioxidant properties. These bioactive compounds are often effectively extracted by methanol, as it can dissolve both hydrophilic and lipophilic antioxidants. Consequently, the moderate DPPH radical scavenging activity observed in this study may be due to the presence of such compounds. However, further analysis to identify and quantify the specific active constituents would offer a more comprehensive understanding of the extract's antioxidant capabilities.

The FRAP assay is another widely used method to assess the antioxidant capacity of plant extracts. Unlike the DPPH assay, which measures the ability to scavenge free radicals, the FRAP assay evaluates the extract's ability to donate electrons and reduce oxidized species, such as ferric ions (Fe^{3+}), to ferrous ions (Fe^{2+}) [49]. This ability to reduce metal ions is a key mechanism through which antioxidants can exert protective effects against oxidative damage.

In this study, the FRAP value of the methanolic extract was recorded at 113.61 µmol Fe^{2+}/g. This indicates a moderate to good ability of the extract to donate electrons and reduce oxidized species, thereby demonstrating significant antioxidant potential. Typically, higher FRAP values are associated with stronger antioxidant activity, as the extract is capable of reducing a greater number of ferric ions. However, the exact level of antioxidant activity will depend on the specific composition of the extract, including the presence of compounds that are particularly effective at reducing ferric ions.

The FRAP value of 113.61 µmol Fe^{2+}/g suggests that the methanolic extract is rich in electron-donating compounds, which contribute to its ability to neutralize oxidative stress. This is consistent with the results from the DPPH assay, as both tests indicate moderate antioxidant activity. It is also important to note that the FRAP assay, unlike the DPPH assay, measures the total antioxidant capacity, encompassing a broader range of antioxidant activities beyond radical scavenging. As a result, the FRAP assay can provide complementary information to the DPPH assay, enhancing the overall assessment of the extract's antioxidant potential.

The DPPH and FRAP assays measure different aspects of antioxidant activity radical scavenging and electron donation, respectively. Despite this difference, the results from both assays in this study suggest that the methanolic extract exhibits moderate antioxidant activity. The DPPH scavenging percentage of 47.21% and the FRAP value of 113.61 µmol Fe^{2+}/g are consistent with each other, reinforcing the conclusion that the extract has a moderate ability to neutralize free radicals and reduce oxidized species.

Conclusion

The study shows that *Acalypha wilkesiana* contains phytochemicals such as alkaloids, phenol, flavonoids, saponins, tannins, glycosides, steroids and coumarins. The methanolic extract of the leaves contain a total phenolic content of 81.627 ± 0.321 mg gallic acid equivalent per gram of extract, a total flavonoid content of 29.988 ± 0.089 mg quercetin equivalent per gram of extract, and a total saponin content of 2.34 ± 0.042%. The high phenolic and moderate flavonoid content indicates that the plant can be used as a natural source for antioxidant and can be used to explore new drug leads.

The GC-MS analysis revealed the presence of 16 compounds which were also identified. Among the compounds identified, 3-Eicocene and Furfural has been reported to possess anti-inflammatory as well as antioxidant properties which help to prevent oxidative stress-related diseases and been used as natural preservative.

The results of the DPPH and FRAP assays in this study indicate that the methanolic extract possesses moderate to good antioxidant activity. The DPPH radical scavenging percentage of 47.21% and the FRAP value of 113.61 µmol Fe^{2+}/g suggest that the extract has a significant ability to neutralize free radicals and donate electrons to reduce oxidized species. These findings align with the results of other studies, although the methanolic extract in this study exhibits lower antioxidant activity than some other extracts, such as those reported by Gupta *et al*., [12].

Overall, the moderate antioxidant activity of the methanolic extract from *Acalypha wilkesiana* leaf suggests its potential as a natural antioxidant source, which could be further explored for therapeutic and preventive applications in the context of oxidative stress-related diseases.

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